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Precomplexation and Activation of Carboxylate and Phosphate Esters

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(1) Statement of objectives

We are looking for reaction types that simultaneously: (1) provide for the reaction of acyl and phosphoryl groups under non-forcing conditions; (2) suggest ways for elaboration into catalytic cycles with turnover behavior; and, (3) survive translation onto binding moieties. To date, we have focussed on artificial metalloenzymes derived from Co(III) and Cu(II) cordination complexes with cyclodextrins, preassociating α-nucleophiles, and binuclear metal ion complexes

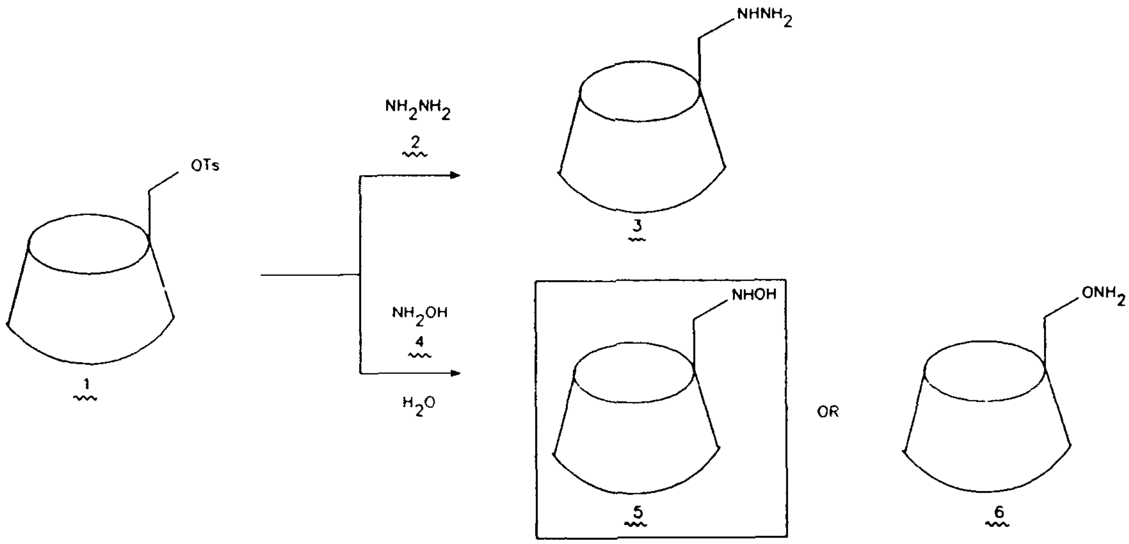
(2) Statement of accomplishments

PREASSOCIATING α-NUCLEOPHILES

Cyclodextrins have been prepared bearing imidazole as a group with reactivity at pH 7; ndant coordination complexes have likewise been employed. However, as potential pendant groups, α-nucleophiles such as hydrazine or hydroxylamine offer unique properties. (1) In solution, α-nucleophiles show enhanced reactivity towards acyl transfer as compared to isosteric alcohols or amines. (2) Despite their greater reactivity towards acyl compounds, hydroxylamine (pK_a 5.97) and hydrazine (pK_a 8.0) are less basic than isosteric amines (pK_a 9-10), and thus exist in a reactive form near neutral pH. (3) Both hydroxylamine and hydrazine transacylate alkyl esters and amides. (4) Because they are physically small, pendant α-nucleophiles would necessarily reside proximal to the CD binding cavity. We now report on the syntheses, characterizations, and reactivities of $\beta CDNHNH_2$ and βCDNHOH.

Reaction of βCD-1^o-tosylate (1) in anhydrous hydrazine (2) at RT for 4 h, followed by precipitation from EtOH, gave the crude product (3). Physically entrained NH_2NH_2 was removed by reprecipitation from EtOH (5x), which gave 3 in 60% yield. In an analogous manner, reaction of 1 with a 6% aqueous solution of hydroxylamine (4) at 90°C for 3 h, followed by multiple reprecipitation from EtOH, gave 5 in 86% yield. While either the N- or the O-alkylation product might have been formed, catalytic hydrogenation, which yielded $\beta CDNH_2$ and not βCD itself, confirmed the former. Notably for an unsymmetrically substituted CD derivative, 5 yields colorless plates (dec 207-210°C) from water.[9]

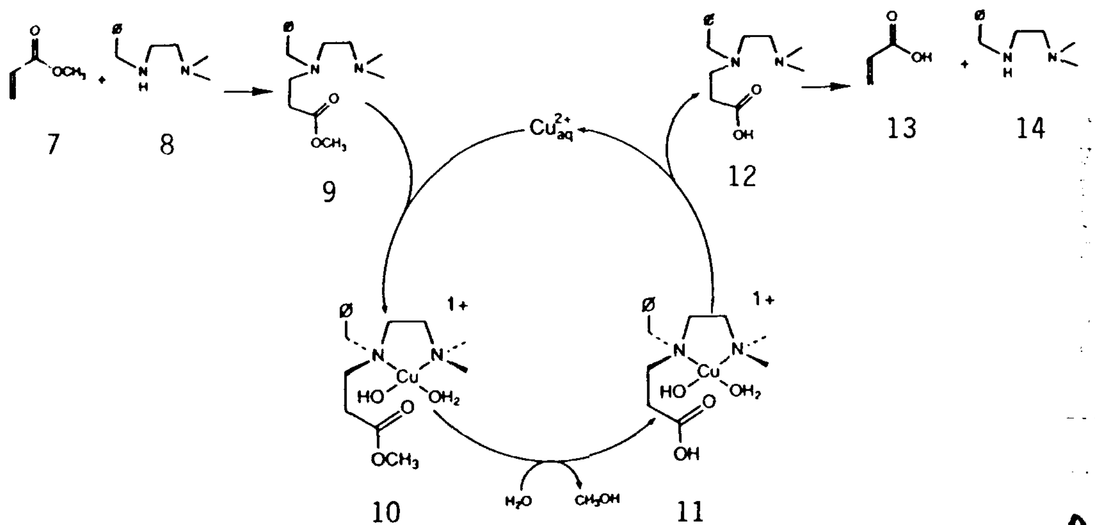
Both $\beta CDNHNH_2$ and βCDNHOH are acylated rapidly by p-nitrophenylacetate (pNPA) with saturation behavior. The reaction of pNPA (0.05 mM) fully complexed to 5 at pH 7.0 and 25°C is faster than that with equimolar CH_3NHOH (k_2=1.0 M^{-1} s^{-1}), demonstrating an effective



RNHOH concentration of 37 mM. βCDNHOH is acylated as efficiently at pH 7.0 as at pH 9.5; furthermore, the rate of acyl transfer is 1500-times faster than that afforded using equimolar βCD, which is not reactive under neutral conditions (pub. 7).

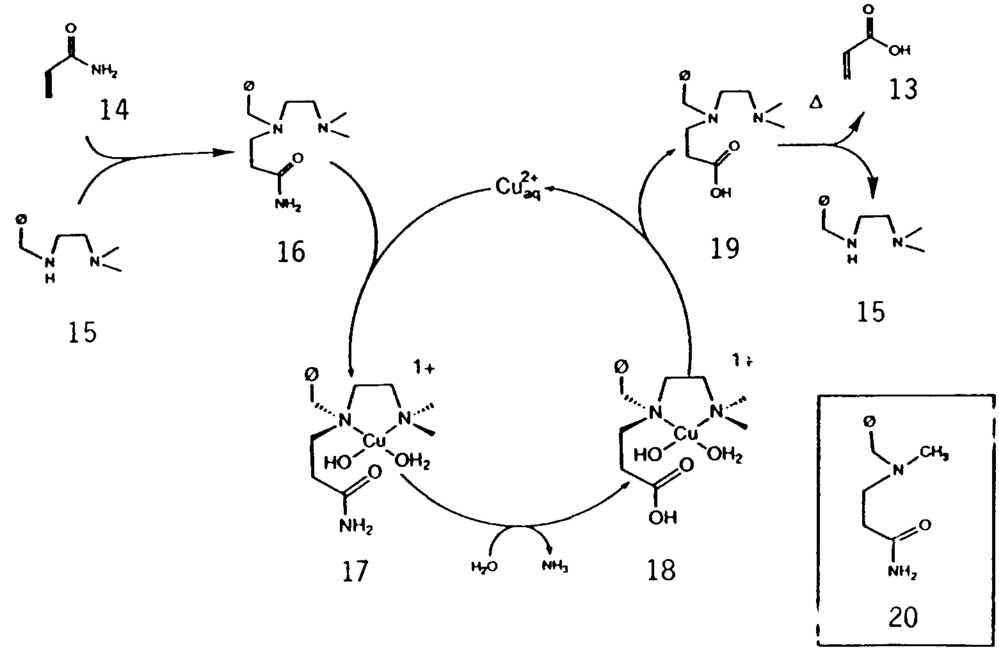
CATALYSIS VIA REVERSIBLE COVALENT BOND FORMATION

Concurrently, we have been investigating the potential of a different mode of reversible complexation: the Michael reaction. Without added metals, the hydrolysis of ester 9 (1.8 mM) to acid 12 proceeded at pH 7.50 and 23°C with k_{obs}=1.0 x 10^{-6} s^{-1} ($t_{1/2}$=670,000 s). Addition of divalent metal ions accelerated the rate of the hydrolysis by varying extents. The addition of 1 eq of Co(II) or Ni(II) accelerated the hydrolysis, yielding k_{obs}=3.9 x 10^{-6} s^{-1} ($t_{1/2}$=170,000 s) and 1.2 x



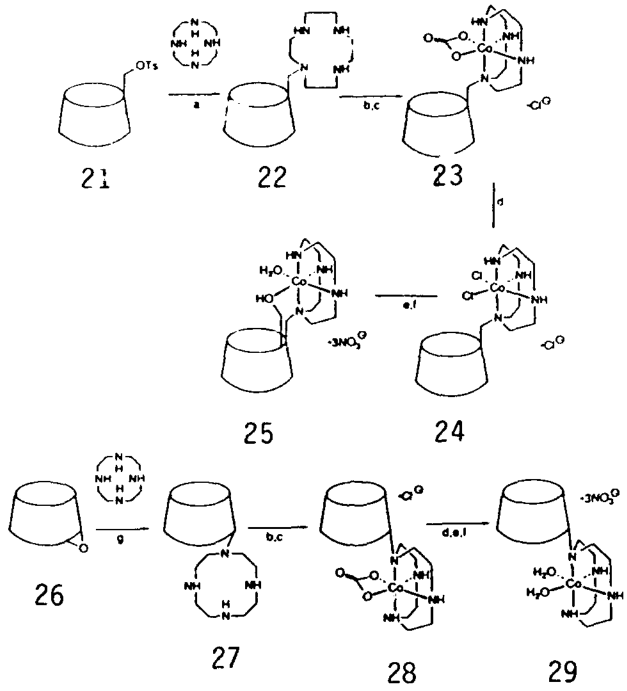
10^{-5} s^{-1} ($t_{1/2}$=57,000 s), respectively. The use of copper ion led to the largest accelerations; addition of 1 eq of Cu(II) gave k_{obs}=6.2 x 10^{-3} s^{-1} ($t_{1/2}$=110 s), and addition of 5 eq of Cu(II) gave k_{obs}=1.6 x 10^{-2} s^{-1} ($t_{1/2}$=44 s). Therefore, the hydrolysis rate for the fully complexed ester is 16,000-times faster than the same reaction without added metal. Significantly, the reaction was catalytic with respect to copper ion; 0.01 eq of Cu(II) gave k_{obs}=6.2 x 10^{-6} s^{-1} ($t_{1/2}$=110,000 s), and the reaction proceeded to >90% completion while following first order kinetics. As we did not observe product inhibition, it must be inferred that the complexation of Cu(II) to acid **12** is readily reversible and largely incomplete at the lower Cu(II) concentrations (**pub.** 2).

Even more encouragingly, the same scheme demonstrates metal-catalyzed hydrolysis of an unactivated amide. Without added metals the hydrolysis of amide **16** to acid **19** is too slow at pH 7.50 to measure readily, but no (≤2%) reaction had occurred after 650 h at 50°. As one basis for comparison, Still has measured the rate of peptide hydrolysis at 25° and pH 6-8 as k_{obs}=3 x 10^{-9} s^{-1} ($t_{1/2}$=ca. 7 years). With 1 equivalent of $Cu(ClO_4)_2$ at pH 7.0 and 23°, the hydrolysis of **16** occurs to at least 97% completion with k_{obs}=2.5 x 10^{-6} s^{-1}. The reaction with 0.5 eq of Cu(II) at pH 7.5 and 50° proceeded to >97% completion and showed biphasic kinetics: $k_{(obs)}$= 8.7 x 10^{-5} s^{-1} (first half) and 4.5 x 10^{-6} s^{-1} (second half). Reference **20** did not hydrolyze under metal catalyzed conditions. While such observations of metal ion promotion of amide hydrolysis have been made before, *the reaction we observe is catalytic with respect to copper ion*. The use of 8 mM **16** and 2 mM Cu(II) (0.25 equivalents) at pH 7.5 and 50° resulted in 80% conversion to acid **19** after 10 days. Likewise, the use of 1.2 mM Cu(II) (0.15 equivalents) at pH 7.0 and 50° resulted in 74% conversion to acid **19** after 15 days. These reactions, while not of enzyme-like speed, represent the first examples of metal catalyzed amide hydrolysis in a synthetic system (**pub.** 5).

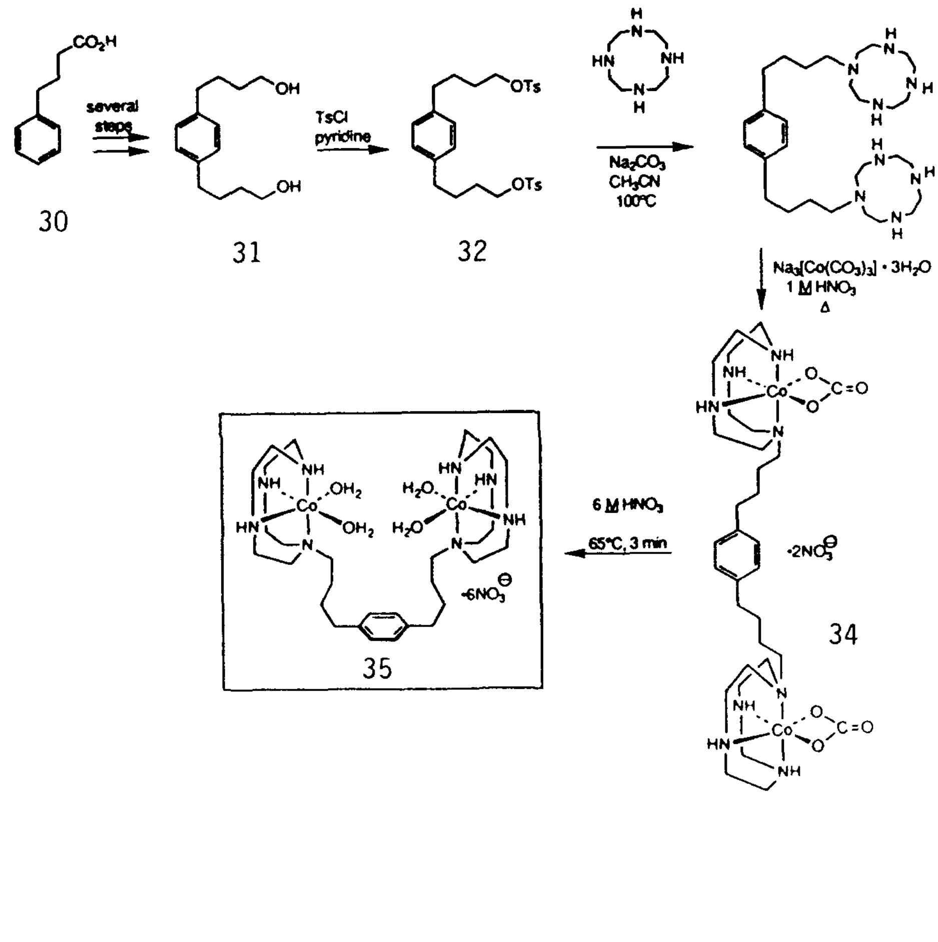


METALLOENZYME MIMICS

We have prepared isomeric cyclodextrin/Co(III)-azamacrocycle conjugates 25 and 29. The primary side derivative (25) increases the rate of p-nitrophenylacetate hydrolysis by a factor of 900-fold; equally significant is the fact that the acceleration is observed at pH 7, at which cyclodextrin itself shows no activity (**pub.** 1,8).



We have also prepared binuclear complex **35**, which accelerates the hydrolysis of the bis(PNP)phosphodiester to a greater extent than two equivalents of the monomeric cyclen-Co(III) complex (**pub. 4**).



(3) List of publications to date resulting from this work

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8) E. U. Akkaya and A. W. Czarnik, "Synthesis and Transacylating Activity of Isomeric Co(III)-Cyclodextrin Artificial Metalloenzymes", *J. Phys. Org. Chem.* **1992**, *5*, 0000.